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Induction of systemic acquired resistance in cotton by BTH has a negligible effect on phytophagous insects

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Abstract

Whether or not chemical changes in plants in response to pests (insects and pathogens) are general or specific remains unclear. Some evidence indicates that an induced response (IR) to arthropods via the octadecanoid pathway represents a distinct mechanism from the salicylic acid-based pathway of systemic acquired resistance (SAR) to pathogens. To further test this hypothesis, young cotton seedlings were activated with benzo (1,2,3) thiadiazole-7-carbothioic acid (S) methyl ester (BTH), an elicitor of SAR. The enzymatic activities of a number of pathogenesis-related (PR) proteins in young and old leaves of control and BTH treated plants were measured. BTH applications elicited marked increases in the activity levels of chitinase, peroxidase, and β-1,3-glucanase both locally and systemically. The highest levels of induction were detected systemically in young leaves. Except for some local effects on whitefly oviposition, the induction of SAR by BTH had no effect on either host preference of whiteflies *Bemisia tabaci* (Gennadius) or on feeding efficiency of cotton bollworms *Helicoverpa armigera* (Hübner). We conclude that SAR induction via the salicylic acid pathway in 'Acala' cotton has negligible effect on the tested insect herbivores.

Introduction

The ability of plants to actively defend themselves with inducible and constitutive mechanisms has been extensively demonstrated in many systems (Karban & Baldwin, 1997). Induced defences against virus, fungi and bacteria are usually referred to as systemic acquired resistance (SAR), whereas defence against insect attack is named induced resistance (IR). A key compound that regulates SAR against pathogens is salicylic acid, which activates gene expression of defencive factors such as pathogenesis-related (PR) proteins (Kombrink & Somssich, 1997).

Induced resistance to insects is often associated with the octadecanoid pathway (i.e., via jasmonic acid) that leads to the production of proteinase inhibitors and secondary metabolites. Some studies have demonstrated an antagonistic relationship between IR and SAR responses. Salicylic and jasmonic acids interfere with each other's production and following defence responses. Salicylic acid inhibits wound-induced proteinase inhibitors (IR) in tomato by blocking jasmonic acid-based gene expression (Doherty et al., 1988; Pena-Cortes et al., 1993; Doares et al., 1995). Simultaneous application of the elicitors reduced the efficacy of each mechanism in reducing pathogens and insect performance (Thaler et al., 1999; see Inbar et al., 1998).

Nevertheless, plant defence mechanisms whether specific or not, are more complex and the dichotomy is not always justified (Maleck & Dietrich, 1999). Large body of evidence indicates that salicylic acid is involved in multiple defence pathways. These pathways may interact with jasmonic acid and ethylene (synergistically or antagonistically) or trigger an independent induced response (Pieterse & van Loon, 1999).

Consequentially, in some plants, the defence mechanisms provide cross-resistance against pathogens and arthropods (McIntyre et al., 1981; Karban et al., 1987; Benedict & Chang, 1991; Kogan & Fischer, 1991; Inbar et al., 1998). Recently, Eichenseer et al. (1999) characterized the activity of glucose oxidase produced in the labial glands of *Helicoverpa zea* larvae. Secretion of this enzyme with insect saliva may activate the SAR system in the host plant against pathogens. However, several studies revealed more specific defences where SAR did not provide protection against arthropods (Apriyanto & Potter, 1990; Ajlan & Potter, 1992; Thaler et al., 1999).

Although the mechanisms are unclear, it appears that induced responses in cotton (*Gossypium hirstum*) are rather general. Boll weevil (*Anthonomus grandis*) survival and host selection were negatively affected by bacterially induced changes in cotton leaves (Benedict & Chang, 1991). Furthermore, indirect (plant mediated) interspecific competition was found between a fungal pathogen (*Verticillium dahliae*) and spider mites (*Tetranychus urticae*) that shared cotton as a host plant. Initial fungal infection reduces cotton suitability (unknown factors) for mites and *vice versa*. These interactions were clearly mediated by cotton induced responses (Karban et al., 1987).

Based on the fact that salicylic acid act in multiple pathways (Pieterse & van Loon, 1999), we investigated whether or not induction of the cotton SAR response could provide protection against insect herbivores (i.e., act as an 'IR function'). We triggered the SAR pathway with a mimic of salicylic acid-benzo (1,2,3) thiadiazole-7-carbothioic acid (S) methyl ester (BTH; Bion®; Actigard®). BTH is commercially sold as an elicitor of SAR in various crops (Kunz et al., 1997). The induction of the salicylic pathway by BTH applications was revealed through the analyses of several PR protein classes known to be induced by salicylic acid. We then examined the effect of BTH-induction on insect performance using two polyphagous species that have different feeding modes. We selected the phloem-feeding whitefly *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae); these whiteflies oviposit and feed on the same leaves (van Lenteren & Noldus, 1990). Also selected were the plant-chewing larvae of the cotton bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), that feed on cotton leaves and bolls (Fitt, 1989).

Materials and methods

Cotton plants (*G. hirstum* cv. 'Acala' SJ2) were grown in 1 L pots in a greenhouse. Once per week, plants were fertilized 20:20:20 NPK. No pesticides were used throughout the experiment. At 16 d of age, the plants were randomly assigned to two groups (total $n = 120$). Half of the plants were sprayed with water (control) and the other half was sprayed to run-off with 0.8 g l^{-1} BTH (Actigard®). This treatment was repeated one week later (age 23 d). Both groups of plants shared the same bench in the greenhouse. The biochemical analyses and insect trials were started two weeks after the second treatment.

Phytochemistry. Fresh leaves #3 and #4 (old), and #7 and #8 (young) from the bottom of the plant (at this stage each plant had 9-10 leaves) were sampled from 16 control and 15 BTH-treated plants. The young leaves grew after the last treatments and, therefore, any changes in their PR protein activity levels in relation to the old leaves were considered as a systemic response. Changes in the old leaves were considered local induction. Samples were weighed, freeze-dried, and then ground in liquid N_2 using an Omni-Mixer (OCI Instruments, Waterbury, CT). The resulting powder was suspended in cold 0.1 M sodium phosphate (pH 7.4) and homogenized for 1 min. The homogenate was filtered through four layers of cheesecloth and centrifuged at 15 000 *g* for 15 min at 4 °C. The supernatant was filtered through a layer of Miracloth (Calbiochem, La Jolla, CA). Filtrates were subjected to desalting (Econo-Pac 10DG, BioRad, Hercules, CA) and lyophilyzed. Chitinase activity was measured colorimetrically using dye labeled chitin (Loewe Biochemica, Munich, Germany) as substrate (Wirth & Wolf, 1990). β-1,3-Glucanase activity was assayed using the method of Abeles & Forrence (1970) with laminarin (*Laminaria digitata*, Sigma, St. Louis, MO) as substrate. Peroxidase activity was measured based on the method outlined in the Worthington Enzyme Manual (Worthington Biochemical Corporation, 1993), using 4-aminoantipyrine as hydrogen donor.

Insect performance. Two leaves (#3 and #7) from the control and BTH treated plants (each $n = 14$) were cut, the leaves were placed individually in Petri dishes, and the petioles were covered with wet cotton wool. A single, third instar larva of *H. armigera* was weighed and placed in each dish. The source of larvae was a colony reared on artificial diet at constant temperature

of 26 °C and L14:D10 at the Volcani Center, Israel as reported previously (Rafaeli & Soroker, 1989). Larvae were kept at room conditions (ca. 25 °C) for three days and then reweighed. Relative growth rate (RGR) was calculated for each larva based on fresh weight. An additional 16 plants from each group were randomly placed on a 1 m-height bench in the middle of a greenhouse that housed a large colony of whiteflies reared on cotton. Whiteflies were allowed to select the experimental plants as feeding and ovipositioning hosts for 24 h. Approximately 8 h after the beginning of the experiment, the plants were shaken and replaced in a random manner. The number of whitefly eggs on two randomly selected 2 cm^2 areas was counted on the sampled leaves (leaves #3 and #7). Since leaves on the same plants are not independent, we used paired t test to compare differences between treatments. The paired *t* test was used to compare values between young and old leaves on the same plants.

Results

Phytochemistry. BTH had a very strong effect, both locally and systemically, on most of the activities of the PR proteins measured. In general, it appeared that systemic induction in young leaves tended to be more pronounced than the local induction in old leaves. There was no detectable lysozyme activity in any of the samples analyzed. Locally, BTH increased the level of chitinase by 3-fold ($t = 5.73$, df = 29, P<0.01). The levels of chitinase in young leaves of BTH-treated plants were 10-fold higher than the levels in the control plants ($t = 5.12$, df = 29, $P < 0.01$, Figure 1). A similar trend was found with β-1,3-glucanase (Figure 2). BTH significantly elicited PR proteins both locally ($t = 4.25$, df = 29, $P < 0.01$) and systemically ($t = 5.05$, df = 29, $P < 0.01$, Figure 2).

The largest level of induction was found with peroxidase (Figure 3). Compared with the controls, peroxidase levels were about 12-fold higher locally ($t = 5.4$, df = 29, P<0.01) and nearly the same systemically ($t = 3.7$, df = 29, P<0.01).

Only with chitinase was a within-plant correlation found between the magnitude of local and systemic induction, i.e., between enzyme levels in young vs. old leaves in the same BTH-treated plant ($r = 0.64$, df = 15, $P < 0.01$).

Insect performance. Irrespective of treatment, whiteflies preferred young to old leaves as oviposition sites

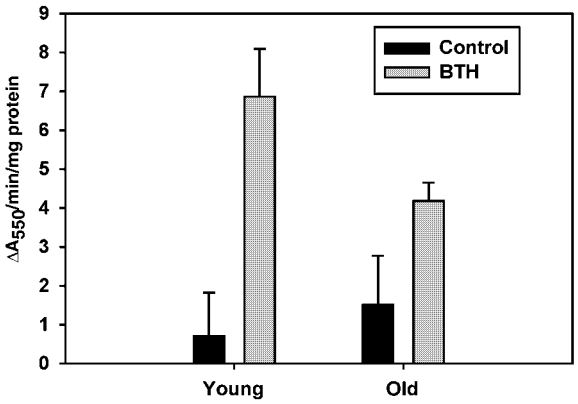

Figure 1. Chitinase activity in control and BTH treated plants. Young leaves that grew after the treatment represent systemic induction. The local and systemic level of activity induction was similar. Note that the systemic induction of chitinase activity in the BTH treated plants was nearly twice the local induction. Data are mean ± se; see text for statistical analysis.

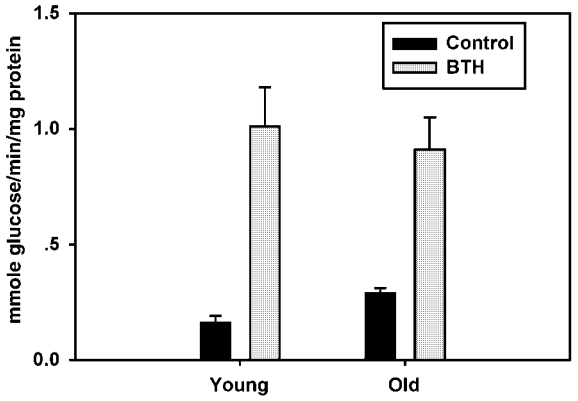

Figure 2. Activity of β-1,3-glucanase in control and BTH treated plants. Young leaves represent systemic induction. Data are mean ± se; see text for statistical analysis.

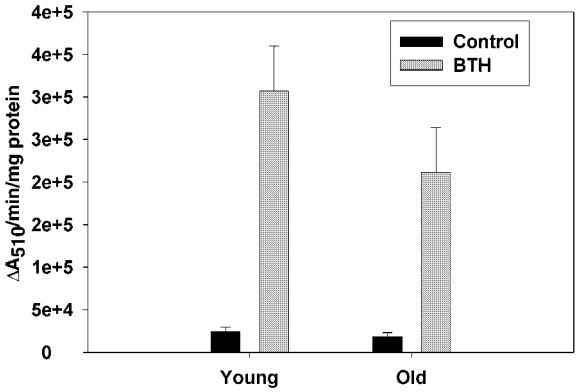

Figure 3. Peroxidase activity in control and BTH treated plants. Young leaves represent systemic induction. Data are mean ± se; see text for statistical analysis

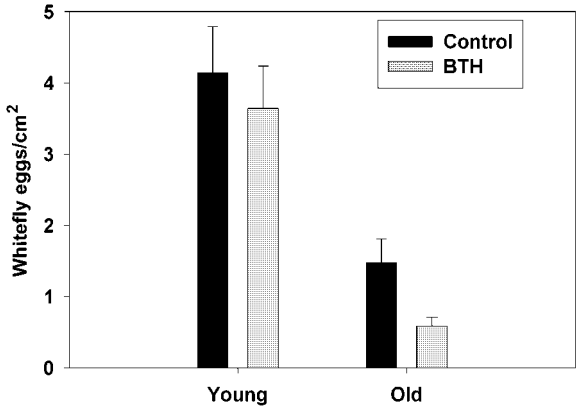

Figure 4. Oviposition site selection by whiteflies. Young leaves are the mean number of eggs on leaves #3 and 4. Old leaves are the mean number of eggs counted on leaves #7 and 8. Regardless of BTH, whitefly preferred young cotton leaves over old leaves as ovipositioning site. Data are mean ± se.

(Figure 4). Approximately 75% of the eggs were found on young leaves (paired $t = 3.75$ and 3.05, df $= 14$, $P < 0.01$ for control and BTH-treated plants respectively). Treatment with BTH had no systemic effect on whitefly host preference ($t = 0.56$, df $= 30$, ns). BTH-induced changes caused ∼60% reduction in egg density on old leaves, indicating some localized effect ($t = 2.54$, df $= 30$, $P < 0.05$, Figure 4). Larval survival of *H. armigera* was about 90% in all trials. The relative growth rate of the caterpillars when feeding on the young leaves was 0.18 ± 0.011 for the BTH-treatment and 0.16 ± 0.019 for the untreated control ($t = 0.86$, df $= 26$, ns). Similar results were found on old leaves where RGR was 0.11 ± 0.008 and 0.12 ± 0.012 for BTH-treated and control leaves respectively ($t = 0.78$, df $= 27$, ns). Thus, BTH-induced changes in cotton had no effect on larval feeding efficiency and survival.

Discussion

BTH is an excellent elicitor of the salicylic acid activated defencive pathway in cotton, inducing remarkable activities of PR-proteins both locally and systemically (Figures 1–3). However, this induction had little effect on the insect herbivores tested, except for a local effect on whitefly oviposition. High levels of chitinase, peroxidase and β-1,3-glucanase in cotton leaves (and probably other phytochemicals induced by BTH that were not measured in this study) have no or little effect on the herbivores tested here. These enzymes play a key role in plant defence systems against pathogens (Kombrink & Somssich, 1997). Chitinase and β-1,3-glucanase are the major components of pathogen cell wall, while peroxidase has a broader function such as H_2O_2 metabolism, lignification and production of secondary metabolites (Bowles, 1990). IR of cotton has various components including high activity of oxidative enzymes, reduced nutritional quality, lignification, and production of secondary metabolites such as chlorogenic acid (Bi et al., 1997). At least one of these components, i.e., peroxidase activity, was induced by BTH but didn't provide significant protection against herbivores. Thus, our data support the hypothesis that SAR and IR are distinct defence mechanisms, as demonstrated by Thaler et al. (1999) who reported that treatment with BTH did not hamper caterpillar survival and even improved thrips performance on tomatoes.

Inbar et al. (1998) tested the efficacy of several elicitors in tomato pest control. BTH provided the best control of several bacterial and fungal pathogens. In addition, Inbar et al. (1998) reported mild and inconsistent effects of BTH-induced changes on insect herbivores. BTH-induced changes reduced the density of the leaf miner (*Liriomyza trifolii*) (adult host preference) in tomatoes but not larval survival. Treatment with BTH had no effect on the population levels of the silverleaf whitefly, *B. argentifolii*, in tomato fields (Inbar et al., 1998). Here, on the other hand, BTH induced local resistance of cotton to whiteflies (Figure 4). Taking into account that the different pathways may not be similarly regulated in all systems (Maleck & Dietrich, 1999), it seems that the negative effect of the SAR induced by BTH on insect herbivores should vary among plant and insect species. Certainly the IR in roots of various citrus rootstock varieties resulting from feeding by larval *Diaprepes abbreviatus* varies with variety (Mayer et al., 1995).

Whiteflies preferred the upper-young cotton leaves as oviposition sites, a pattern that was also observed in other studies on many host plants (e.g., van Lenteren & Noldus, 1990; Inbar et al., 1999). SAR induced by BTH did not alter this pattern. The only effect of BTH-induction on herbivores was found locally in old cotton leaves. The reduced whitefly oviposition on old BTH treated plants was quite surprising since the absolute and the relative (compared with the controls) activity levels of most PR proteins measured were higher in the younger leaves. Therefore, we do not know what caused the reduction in whitefly eggs den-

sity on old BTH-treated leaves, but the overall effects on whitefly densities are negligible.

In most studies of cotton that documented efficient induced defences (SAR and IR) for a broad spectrum of pests: mites, insects, fungi and bacteria, the organisms themselves provided the stimuli (e.g., Karban & Carey, 1984; Karban et al., 1987; Benedict & Chang, 1991; Alborn et al., 1996; Wool & Hales, 1996). Artificial induction of cotton IR by artificial damage like clipping provided mix result (Karban, 1985; Anderson & Alborn, 1999). Apparently the high level of production and release of defencive volatiles induced in cotton was not affected by artificial damage (Anderson & Alborn, 1999) and was greatly dependent on the caterpillars' oral secretions (Paré & Tumlinson, 1997). Similarly, volatiles emitted by jasmonic acid-treated Lima beans do not provide the same chemical and biological (attracting carnivorous mites) effects as those induced by herbivorous spider mites (Dicke et al., 1999). Therefore it is possible that triggers provided directly by the organisms stimulate the production of factors that promote SAR and IR which results in broad and cross-resistance, stimuli that may not be mimicked by intermediate agents like BTH. At this time, it appears that cotton resistance induced by the pest organisms themselves is more efficient than resistance resulting from artificial triggers, like clipping, or commercial agents like BTH. Indeed, in their recent review Maleck & Dietrich (1999) concluded that the exogenous application of elicitors may not necessarily reflect the events that follow biological induction in terms of affected organs, concentration and timing.

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